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Simultaneous determination of inorganic anions and cations by supercritical fluid chromatography using evaporative light scattering detection

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ABSTRACT

Supercritical fluid chromatography (SFC) is commonly used for the analysis of non-polar compounds, but remains poorly explored for the separation of polar and ionized molecules. In this paper, SFC has been investigated for the separation of 14 inorganic ions sampled in aqueous solutions. Four polar stationary phases were first screened using CO_2-methanol-based mobile phases containing water or different acidic or basic additives, in order to select the most efficient conditions for the simultaneous retention of inorganic cations and anions and to favor their detection using evaporative light scattering detector (ELSD). Orthogonal selectivity was obtained depending on the stationary phase used: whereas anions are less retained on HILIC stationary phase, 2-ethylpyridine (2-EP) stationary phase exhibits strong interaction for anions. Best results were obtained under gradient elution mode using a 2-EP stationary phase and by adding 0.2% triethylamine in the CO_2-methanol-based mobile phase. The composition of the injection solvent was also investigated. The results showed that a methanolic sample containing a percentage of water not exceeding 20% does not affect the analytical performances obtained on 2-EP. Moreover, the presence of triethylamine in the injection solvent contributes to eliminate peaks shoulders. Among the 14 inorganic ions tested, three cations (Li^+, Ca^{2+} and Mg^{2+}) and five anions (Cl^-, Br^-, NO_3^-, I^-, SCN^-) were totally resolved in 15 min. NO_3^- and NO_2^- still coeluted in the final optimized conditions. The other investigated ions were either strongly retained on the stationary phase or not detected by the ELSD.

1. Introduction

For several years, supercritical fluid chromatography (SFC) has been gaining interest because of the typical properties of the mobile phase, the low cost of carbon dioxide (CO_2) compared to organic solvents, the fast analysis speed, the wide polarity compatibility and also the current development in SFC instrumentation and stationary phases [1,2]. Several recent reviews reported various applications in the field of enantiomeric separations [3,4], drug discovery [4,5] and food analysis [6]. Even if most applications are dedicated to compounds of low or medium polarity, the interest of SFC for the analysis of polar compounds was also demonstrated [7,8]. It requires the use of a polar stationary phase to retain them and a polar mobile phase to ensure their solubility. Several types of stationary phases classically used in liquid chromatography are suitable to retain polar compounds in SFC. These include bare silica [9], diol [10], aminopropyl [11], cyanopropyl [12] and HILIC columns [13]. Other phases (2-ethylpyridine (2-EP), 4-ethylpyridine, propyl-pyridyl urea) have been specifically developed for SFC applications. Among all these columns, 2-EP was highlighted for the analysis of pharmaceutical compounds specifically for basic drugs [14].

Although the addition of a modifier in the CO_2 mobile phase tends to promote the analyte elution, the use of an additive in the mobile phase is a necessity to obtain acceptable chromatographic results in terms of efficiency and peak symmetry [15]. In general, acidic additives can be employed to improve peak symmetry of acidic analytes, whereas basic additives have shown interesting results for basic drugs [16]. Even if it seems to be clear that the additive contributes to increase the solubility of analytes, different hypothesis have been proposed to explain the effect of additives on the analyte retention. The first one suggests that additive can interact with the active sites (for example silanol groups) of the stationary phase in order to limit adsorption of the analytes. Another

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explanation involves the formation of an adsorbed additive monolayer which may interact with polar solutes through an ion pair type mechanism [17]. Finally, some other authors suggest that additives act by suppressing the analyte ionization in the mobile phase either by modifying the apparent pH of the mobile phase or thanks to the formation of ion pairs between the additive and the ionic solute [18]. In fact, the role of the additive and the action mechanism is not clearly elucidated. Hence, several basic drugs with pKa values ranging from 8.3 to 10.3 were successfully resolved on 2-EP stationary phase using methanol as co-solvent and trifluoroacetic acid as additive [8]. Likewise, sulfonate derivatives were surprising eluted as symmetrical peaks on silica column by adding lithium acetate in the methanol co-solvent [17].

Since the chromatographic behavior of ionic compounds cannot be predicted as a function of the additive used in the co-solvent, the strategy performed by different authors is to screen various additives during the development of a new SFC method dedicated to the analysis of polar and/or ionizable compounds [19].

The determination of inorganic ions is of great importance because the fields of applications are very large. Indeed, food and pharmaceutical industries, environmental and medical sectors need sensitive and high throughput techniques to analyze the huge number of samples. Since the 90′s, ion-exchange chromatography is the technique of choice for the analysis of inorganic ions due to the development of robust apparatus and the description of well-known methods in the literature [20]. The separation of inorganic anions was also investigated in normal phase chromatography using diol [21] and porous graphitic carbon stationary phases [22], but to our knowledge none method has been developed in SFC. Capillary electrophoresis (CE) is the second technique the most employed to resolve inorganic ions using conductivity, fluorescence or indirect UV detection [23,24]. The low sample consumption and the fast analysis constitute the two main advantages of CE compared to ionic chromatography. Despite the performances of these techniques, a few methods have been developed for the separation of inorganic anions and cations in a single run: only three papers deal with the simultaneous separation of inorganic anions and cations by CE based on the double sample injection on both sides of the capillary [25], by reversed phase liquid chromatography using an ion-pairing agent [26] or by ionic chromatography [27].

When chromatography is used, regarding the chemical structure of ions, detection is often performed using a conductivity detector [28], chemiluminescence or UV at a low wavelength [20]. It is noteworthy that evaporative light scattering detection (ELSD) was successfully used in several studies to detect inorganic ions although is normally applied for analytes which exhibit molecular mass larger than 100. Hence, ELSD was employed for the detection of inorganic cations such as Na^+ [27,29,30], K^+, Mg^{2+} and Ca^{2+} [27,30] or anions like Cl^- [30], NO_3^- [27,31], Cl^- and SO_4^{2-} [31], $H_2PO_4^-$, Br^-, ClO_3^-, I^- and ClO_4^- [22]. In all cases, hydro-organic mobile phase was supplemented with an additive like ammonium formate [27] or ethylene diammonium formate [30], trifluoroacetic acid [29], formic acid and pyridine [22] or an ion pairing agent such as pentylammonium or heptylammonium formate [31]. Generally, the mechanism allowing the detection of inorganic ions by ELSD was not discussed by the authors. Elfakir et al. [22] underlined that the presence of pyridine in the mobile phase enhanced the ELSD response of several anions and Mercier et al. suggested an ion pairing mechanism to explain both the retention and the detection of anions [31].

The aim of this study was to investigate, for the first time, the potential of SFC-ELSD to analyze simultaneously a pool of 14 inorganic ions (6 cations and 8 anions). The first part presented in this paper will be dedicated to the selection of the stationary phase and the composition of the mobile phase to ensure both good chromatographic profiles and suitable ELSD response. In the second part of this article, attention will focus on the composition of the injection solvent to obtain symmetrical and efficient chromatographic peaks. Finally, several validation criteria will be examined to verify if the proposed method could be applied to quantitative applications.

2. Materials and methods

2.1. Chemicals and solvent additives

Potassium iodide (KI), sodium nitrite ($NaNO_3$), diethylamine (DEA), triethylamine (TEA), *n*-butylamine, trifluoroacetic acid (TFA), ethanesulfonic acid ($EtSO_3H$), ammonium acetate, perfluorooctanoic acid (PFOA) and pentafluoropropionic acid (PFPA) were purchased from Sigma Aldrich (Steinheim, Germany). Potassium thiocyanate (KSCN), sodium sulfate (Na_2SO_4), magnesium chloride ($MgCl_2$), lithium bromide (LiBr), calcium chloride ($CaCl_2$), sodium nitrite ($NaNO_2$), zinc chloride ($ZnCl_2$) and sodium hydrogenophosphate (Na_2HPO_4) were obtained from Merck (Fontenay-sous-bois, France). All solvents used (MeOH, EtOH, 2-PrOH, ACN) were of analytical grade and were provided by VWR (Val de Fontenay, France). The carbon dioxide of N45 quality was purchased from Linde Gas (Rouen, France). Ultra-pure water was supplied by a Milli-Q purification system from Millipore (St Quentin en Yvelines, France).

2.2. Chromatographic apparatus

Chromatographic separations were carried out using a SFC-PICLAB hybrid 10–20 apparatus equipped with an autosampler, three 40P pumps, a column oven with a 10-columns selection valve and a 6-solvents switching valve (PIC solution, Avignon, France). The proportion of the co-solvent in the mobile phase was adjusted by a piston pump. It was then directly added in the carbon dioxide feeding, and the mixture of co-solvent and carbon dioxide was pumped by another piston pump at the total flow-rate. The pump head used for CO_2 was cooled to −8 °C by a cryostat (Huber Minichiller, Offenburg, Germany). The injection valve was supplied with a 20 μL sample loop. The unit was also composed of a Smartline 2600 DAD detector with a high-pressure resistant cell (Knauer, Berlin, Germany). Detection wavelength was set at 220 nm. After the detector, the outlet pressure was controlled by a back-pressure regulator (BPR). The outlet tube was heated at 55 °C to avoid ice formation during the carbon dioxide depressurization. An ELSD model Sedex 85 (Sedere, Alfortville, France) was used in this study. It was plumbed between the PDA and the back pressure regulator using a 0.010 inch i.d. stainless steel tee-junction and a 65 μm × 160 cm Peek tubing. During the separation optimization, the pressure of the nebulizer gas (N_2) was set at 3 bar, the drift tube temperature and the gain were 50 °C and 8, respectively. Data were recorded with SFC PicLab Analytic Online 3.1.2 and processed with Analytic Offline 3.2.0.

2.3. Columns

The columns tested were a Viridis 2-Ethylpyridine (2-EP; 250 mm × 4.6 mm, 5 μm) and a Viridis Silica (Silica; same dimensions) from Waters Europe (Guyancourt, France), a Luna HILIC (HILIC; 250 mm × 4.6 mm, 5 μm) and a Luna NH_2 (Amino; same dimensions) from Phenomenex (Le Pecq, France). The column temperature was set at 35 °C and the outlet pressure at 150 bar. The mobile phase was delivered at a flow rate of 3 mL min^{-1}. A generic gradient from 5 to 30% of co-solvent in 20 min was used to elute analytes, unless otherwise specified.

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